1 **The *Bacillus subtilis tyrZ* gene encodes a highly selective tyrosyl-tRNA**

2 **synthetase and is regulated by a MarR regulator and T box riboswitch**

3 Rebecca N. Williams-Wagner[a], Frank J. Grundy[a], Medha Raina[a, b], Michael Ibba[a, b], and

4 Tina M. Henkin[a, b]

5 **Supplemental Materials**

6 **RNA purification and RT-PCR**

7 *B. subtilis* cells were grown to mid-log phase ($A_{595}$ 0.6-0.8). Cells were harvested

8 and RNA was extracted by the hot acid phenol method (Collart & Oliviero, 2001). To

9 reduce DNA contamination, the RNA was treated with RNase-free DNase I (Turbo

10 DNA-free kit; Ambion) per the manufacturer's instructions. Thermoscript reverse

11 transcriptase PCR (RT-PCR) system (Invitrogen) was used to carry out reverse

12 transcription (4 µg total RNA from BR151 derivatives; 6 µg total RNA from 3610

13 derivatives) using primer tyrZRT (0.5 µM) at 53˚C per the manufacturer's instructions.

14 PCR was performed using Taq polymerase (Invitrogen) and the oligonucleotide primers

15 tyrZRT (RC) and tyrZupstream (Table S2) under the following conditions: 95˚C for 3 m,

16 followed by 40 cycles of 94˚C for 30 s, 60˚C for 30 s, and 72˚C for 60 s. An 850 bp

17 band product is expected from this PCR reaction to indicate expression of *tyrZ*.

18 Reactions to control for DNA contamination were performed in which reverse

19 transcriptase was omitted, and no bands were observed after PCR analysis.

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22 References

23 1. **Collart M, Oliviero S.** 2001. Preparation of yeast RNA. Curr. Prot. Mol. Biol.
24 **13.12**: 1-5.

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**Table S1. Bacterial strains**

| Bacterium | Strain | Genotype | Source/Reference |
|---|---|---|---|
| *B. subtilis* | BR151 | *lys-3 metB10 trpC2 dtd* | Laboratory Stock |
| | ZB307A | SPβ*c2del2*::Tn917::sPK10Δ6 *dtd* | P. Zuber |
| | ZB449 | *trpC2 pheA1 abrB703* SPβ cured *dtd* | P. Zuber |
| | BR151-ZKO | *lys-3 metB10 trpC2 tyrZ::neo dtd* | Grundy *et al*., 1998 |
| | BR151-SKO-EΩ4 | *lys-3 metB10 trpC2 tyrS::neo ywaE*Ω4 *dtd* | This study |
| | BR151-SKO-$E^{opA7G}$ | *lys-3 metB10 trpC2 tyrS::neo* $ywaE^{opA7G}$ *dtd* | This study |
| | BR151-EΩ4 | *lys-3 metB10 trpC2 acsA::cat ywaE*Ω4 *dtd* | This study |
| | BR151-$E^{opA7G}$ | *lys-3 metB10 trpC2 acsA::cat* $ywaE^{opA7G}$ *dtd* | This study |
| | NCIB3610 | *dtd* | BGSC [a] |
| | 3610-ZKO | *tyrZ::neo dtd* | This study |
| | 3610-SKO-$E^{V164E}$ | *tyrS::neo* $ywaE^{V164E}$ *dtd* | This study |
| | 3610 Δ*epsH* | *epsH::tet dtd* | R. Losick |
| | 3610 Δ*tapA-sipW-tasA* | *(tapA-sipW-tasA)::spc dtd* | R. Losick |
| *E. coli* | DH5α | *Φ80dlacZΔM15 endA1 recA1 hsdR17*$(r_K^- m_K^+)$ *thi-1 gyrA96 relA1 Δ(lacZYA-argF)U169* | Bethesda Research Laboratories |
| | BL21(dE3) | *lon ompT* | Novagen |
| | XL2-Blue | *endA1 supE44 thi-1 hsdR17 recA1 gyrA96 relA1 lac* [F´ *proAB* $lacI^q$*ZΔM15* Tn*10* ($Tet^r$) Amy $Cam^r$] | Agilent Technologies |

[a] BGSC, Bacillus Genetic Stock Center, The Ohio State University, Columbus, OH 43210

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## Table S2. Oligonucleotide primers

| Primer | Sequence [a, c] | Use |
|---|---|---|
| **ywaEBamUS** | GAAGTGCGGATCCACCACCTGTCCGTGGCG | Forward PCR primer for construction of *ywaE-tyrZ-lacZ*, *ywaE*$^{opA7G}$*-tyrZ-lacZ*, *ywaE-lacZ*, *ywaE*$^{opA7G}$*-lacZ*, *ywaE-*tyrZΔ*term-lacZ*, and *ywaE*$^{opA7G}$*-tyrZ*Δ*term-lacZ* fusions. Introduces a BamHI site 384 bp upstream of the *ywaE* start codon. |
| **tyrZDS (RC)** [b] | ATGACTTCTAGAGCGCCTGTCATGTATAGC | Reverse PCR primer for construction of *ywaE-tyrZ-lacZ* and *ywaE*$^{opA7G}$*-tyrZ-lacZ* fusions. Introduces an XbaI site 78 bp downstream of the *tyrZ* start codon. |
| **ywaEXbaDS (RC)** | GTTTGAGTCTAGAAAGTGTATCGTGTTCTTC | Reverse PCR primer for construction of *ywaE-lacZ* and *ywaE*$^{opA7G}$*-lacZ* fusions. Introduces an XbaI site 55 bp downstream of the *ywaE* start codon. |
| **tyrZΔtermXb (RC)** | CCATCTAGATAAGGGACGTGGCTCAATGCAC | Reverse PCR primer for construction of *ywaE-tyrZ*Δ*term-lacZ* and *ywaE*$^{opA7G}$*-tyrZ*Δ*term-lacZ* fusions. Introduces an XbaI site 42 bp upstream of the *tyrZ* start codon, removes the 3' half of the *tyrZ* T box transcriptional terminator. |
| **tyrZNcoUS** | ATATTACCATGGATGAGAACATTTGAGCAGCTC | Forward PCR primer to amplify the *tyrZ* coding region. Introduces an NcoI site immediately upstream of the *tyrZ* start codon. |
| **tyrZXhoDS (RC)** | TAATTACTCGAGTTGGAGCTTTAAAAACTTGCG | Reverse PCR primer to amplify the *tyrZ* coding region. Introduces an XhoI site immediately before the *tyrZ* stop codon. |
| **tyrSNcoUS** | ATATTACCATGGATGACTAACTTACTTGAAGACTTA | Forward PCR primer to amplify the *tyrS* coding region. Introduces an NcoI site immediately upstream of the *tyrS* start codon. |
| **tyrSXhoDS (RC)** | TAAAATCTCGAGTTTATACGTCACAAGGAAGTA | Reverse PCR primer to amplify the *tyrS* coding region. Introduces an XhoI site immediately upstream of the *tyrS* stop codon. |
| **dtdBamUS** | TAAGAGGATCCCCAGGCAGTGAATGAAACG | Forward PCR primer used to generate the upstream *dtd* fragment and the final *dtd* gene product. Introduces a BamHI site 196 bp upstream of the *dtd* start codon. |

| | | |
|---|---|---|
| **dtdAUG (RC)** | CTGAACAACTAATCTCATTTCTAACCCCTTTAG | Reverse PCR primer used to generate the upstream *dtd* fragment. Introduces the AUG start codon in place of the AAG lysine codon. |
| **dtdAUG** | CTAAAGGGGTTAGAAATGAGATTAGTTGTTCAG | Forward PCR primer used to generate the downstream *dtd* fragment. Introduces the AUG start codon in place of the AAG lysine codon. |
| **dtdXbDS (RC)** | TTCATATCTAGAAAAAGTAACAGATGGGATCG | Reverse PCR primer used to generate the downstream *dtd* fragment and final *dtd* gene product. Introduces an XbaI site 81 bp downstream of *dtd* stop codon. |
| **ywaEL101E** | AGCAACGTGATCAAAACGGAAGAAAAAAAGAGTTTTTGCCGT | Forward PCR reaction primer used for oligomutagenesis to generate the *ywaE*L101E-*tyrZ*-lacZ fusion. |
| **ywaEL101Erc (RC)** | ACGGCAAAAACTCTTTTTTTCTTCCGTTTTGATCACGTTGCT | Reverse PCR reaction primer used for oligomutagenesis to generate the *ywaE*L101E-*tyrZ*-lacZ fusion. |
| **tyrZRT** | GCTGATCCGTTCCGCC | Reverse primer used for cDNA synthesis of the *tyrZ* gene by reverse transcriptase. |
| **tyrZRT (RC)** | CAAAGTCGTCGCGCTCCATCAGGC | Reverse PCR primer used to amplify the *tyrZ* cDNA. |
| **tyrZupstream** | GGGGAAACAGAGCTCATTGCGGGAA | Forward PCR primer used to amplify the *tyrZ* cDNA. |

[a] Restriction enzymes sites are underlined

[b] RC indicates primers oriented in the reverse complement to the gene sequence

[c] Changes to the gene sequence are double underlined

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**Table S3. Expression of *ywaE-tyrZ-lacZ* fusions**

| | | | β-galactosidase activity | | |
|---|---|---|---|---|---|
| **Fusion** | **Strain** | ***tyrS/tyrZ*** | **- 4ALP** | **+ 4ALP** | **Induction (Fold)** [b] |
| ***ywaE-tyrZΔterm-lacZ*** | SKO-$E^{opA7G}$ | *tyrZ* | 0.83 ± 0.04 | 0.91 ± 0.02 | 1.1 |
| ***ywaE$^{opA7G}$-tyrZΔterm-lacZ*** | BR151 | Both | 24 ± 1.5 | 21 ± 1.8 | 0.87 |
| | SKO-$E^{opA7G}$ | *tyrZ* | 31 ± 4.9 | 32 ± 6.2 | 1.0 |
| | EΩ4 | Both | 57 ± 1.7 | 44 ± 8.2 | 0.77 |

33 [a] Cells harboring each fusion were grown in the presence or absence of 4-amino-L-
34 phenylalanine (4-ALP) until late exponential phase, and then assayed for β-
35 galactosidase activity (Miller units). The values reported are averages of three repeats
36 ± standard error.

37 [b] Induction by 4-ALP was determined by the ratio of β-galactosidase activity in the
38 presence of 4-ALP to activity in the absence of 4-ALP.

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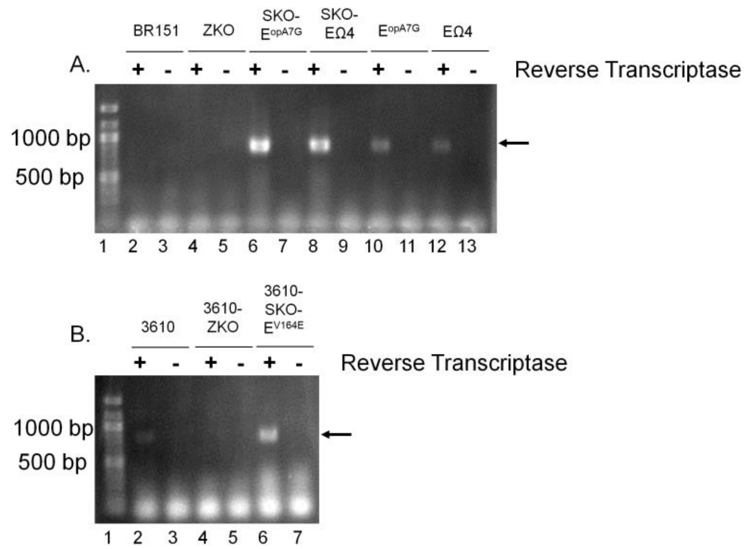


43 **Figure S1: RT-PCR analysis of *tyrZ* expression**

44 RT-PCR was conducted using 4 µg of total RNA from A). BR151 derivatives, and 7 µg total

45 RNA from B). 3610 derivatives. A band of 850 bp was expected to represent expression of

46 *tyrZ*, indicated by an arrow. The strains from which RNA was isolated are indicated above the

47 lanes.

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